**Dong quai**

NTP has proposed extensive toxicity testing of dong quai, a Chinese herbal remedy used primarily to treat conditions of the female reproductive system, despite thousands of years of use in China with no obvious toxic effects. It should also be noted that dong quai is "generally recognized as safe" by the FDA. The NTP's Chemical Information Review Document details a significant body of basic, clinical, and toxicological science for dong quai. There is already existing animal data on most of the proposed areas of testing including ADME, acute toxicity, subchronic toxicity, cytotoxicity, anti-carcinogenicity, reproductive and teratological effects, synergistic and antagonistic effects with other chemicals, and endocrine effects—making it difficult to understand why more testing in some of these areas is proposed.

In addition to relevant information on apical toxicity endpoints, numerous molecular effects of dong quai have also been documented. We believe, given the amount of existing data on the biological activity of dong quai, that the logical course of action is formulation and testing of specific hypotheses designed to further elucidate the effects reported in humans, animals, and *in vitro* – not to replicate them in animals as the test plan proposes.

Besides pathway-based, *in vitro* mechanistic work, a clinical and epidemiologic approach should also be taken with dong quai. Because this is a substance voluntarily taken by a considerable number of people on a regular basis, it presents a unique opportunity for generating such data. A human-relevant approach would allow for investigation of adverse effects previously reported in efficacy studies that can not be easily evaluated in animals including headaches, worsened premenstrual symptoms, and hot flashes. It would also provide an opportunity to explore the potential for genetic differences within the population to impact the effects of dong quai. Greater inclusion of clinical and epidemiological data is in keeping with several of the goals outlined in the NIEHS Strategic Plan for 2006-2011 including: Goal I ("Expand the role of clinical research in environmental health sciences"); Goal IV ("Improve and expand community based research") ; and Goal VII ("Foster the development of partnerships between NIEHS and other NIH institutes…").

The following comments are specific to the test methods proposed:

**Hershberger and Uterotrophic Assays for Material Selection**

Proper materials selection is an important cornerstone of this test plan because different parts of the plant are used (roots and leaves) and these can be processed using a wide variety of techniques (dried, boiled, fried in many different liquids ranging from wine to vinegar and ginger juice), to be sold in different forms (tablet, capsule, liquid extract, tincture, decoction, raw root, essential oil, dried leaves) both alone and in combination with other herbal remedies. Therefore, different preparations of dong quai are likely to have different compositions which could generate differences in toxicity. Incredibly, the plan proposes use of the Hershberger and uterotrophic assays for screening dong quai preparations in order to select appropriate test material. We strenuously object to this proposal because the field of candidate test materials is far too broad to justify the use of 18 rats per test material for the uterotrophic assay and 18-36 rats per test material for the Hershberger assay. Additionally, dong quai has been reported to demonstrate varied and sometimes conflicting estrogenic effects both *in vivo* and *in vitro*; given the equivocal nature of the existing data, we question the use of this endpoint and these assays as a basis for material selection.

Ensuring proper selection of test material requires bioactivity profiling using high-throughput *in vitro* screening, such as with assays of the HTS program at NTP. These assays cover pathways critical to various toxicity endpoints of interest for dong quai including estrogen and androgen receptor binding and gene activation, carcinogenicity, and reproductive and developmental toxicity. Beyond increased efficiency, reduced cost, and replacement of animals, use of the NTP's HTS assays will address potential endocrine effects, as well as a broad universe of other potential pathways of interest that the Hershberger and uterotrophic assays will not. Materials selection should begin with *in vitro* assays to screen dong quai preparations. These results would then be evaluated to inform materials selection and help guide targeted testing if additional *in vivo* data are perceived to be required.

**Acute and Subchronic toxicity**

While the NTP Research Concept document (incorrectly) states that there are no subchronic studies available in the literature, the Chemical Information Review Document states that "[a]cute toxicity studies indicate that administration of dong quai produced no effects at a dose up to 5000 mg/kg; similar results were observed in subchronic studies." The intention to perform 14-day subacute and 90-day subchronic studies is appalling given the existence of this toxicity data. Also, a long history of human use with no reports of acute or subchronic toxicity should be sufficient to preclude further testing.

**Reproductive and Developmental Effects**

We question the necessity of additional rodent assays for reproductive and developmental endpoints given existing studies for these endpoints. Existing mouse data show no effects on fertility, and in fact one study cited in the Chemical Information Review Document showed improved sperm activity. Dong quai also had a positive impact on teratological parameters including protective effects on cartilage and differentiation of neural stem cells in hypoxic embryonic rats and increased placental blood flow and improved fetal development in hypertensive rats.

If additional testing is conducted, NTP should consider conducting an assay according to OECD Test Guideline 422, which combines repeat dose, and reproductive/developmental endpoints. As use of this Test Guideline generates repeat dose data, this would preclude separate subchronic testing (discussed above).

In terms of molecular mechanism, there are conflicting reports on the estrogenic effects of dong quai and other potential interactions with the endocrine system. These questions are best elucidated using *in vitro* biochemical assays, such as receptor binding and transcription activation assays for downstream effects, not the rodent assays proposed.

**Carcinogenicity**

In order to test the carcinogenic potential of dong quai, 2 year bioassays in both male and female rats and mice are proposed. We ask that NTP take a tiered approach to carcinogenicity testing, by beginning with in vitro mutagenicity assays prior to initiation of any in vivo work. Use of the Salmonella mutagenesis assay is mentioned in the test plan and we suggest that this and other in vitro assays such as those used to assess caspases and cyclins in the ToxCast Program (Dix, D.J. et al. 2007), could be used as a screening battery. If the results of the battery are consistently negative, then no further testing should be required.

If rodent bioassays are pursued, we request that the proposal to perform these studies in both rats and mice and in both sexes be reconsidered. Recent EPA work suggests that a "reduced protocol " using a combination of male rats and female mice should be considered as a strategy for reducing testing while adequately determining carcinogenic potential and capturing species differences in tumor types (Rowland, et al. 2009). NTP scientists have also reported that a reduced protocol "using male rat and female mouse would have identified correctly 95 percent of the positive or no evidence chemical carcinogenicity results obtained using the more extensive protocol" (Huff J.and Haseman J. 1991).

**Immunotoxicity and Phototoxicity**

The intended methods for evaluating immunotoxicity and phototoxicity are not specified, making comment on the specifics of the plans difficult. However, there is little data indicating phototoxic or immunotoxic effects. One component of dong quai has been implicated in phototoxicity-bergapten, a furocoumarin found in citrus fruits, parsnips, carrots, and celery. The Chemical Information Review Document indicates that the European Medicines Agency (EMEA), found that bergapten presented no significant risk for phototoxicity when ingested via dietary supplement at up to 1.5 mg/day. Prior to any phototoxicity testing, the composition of the test material selected should be analyzed to determine whether bergapten exposure is likely to exceed 1.5 mg/day.

There is some evidence of skin irritation and other allergic responses, primarily reported as side effects by individuals using dong quai. The magnitude of these responses in humans may be best examined and understood through clinical studies and *in vitro* methods to assess cytokine profiles and effects on other immunomodulatory molecules.

**Synergistic and Antagonistic Effects**

Although there is significant concern over synergistic and antagonistic effects of dong quai, this is not addressed by the proposed experiments. This is a curious omission given numerous reports of interactions with various human drugs as listed in the Chemical Information Review Document. Drug-drug interaction (DDI) data for dong quai is probably the most practical and readily usable data for protection of public health. We suggest considering *in vitro* ADMET studies to screen the litany of drugs suspected of DDI with dong quai (Li, A.P. 2007).

In conclusion, the testing proposed for dong quai is excessive given numerous studies in humans which indicate no serious toxic effects. The Chemical Information Review Document for dong quai mentions single, anecdotal reports of certain adverse effects and reports of relatively mild side effects; these reports do not justify the studies planned. We would also like to point out that dong quai was nominated for this comprehensive toxicological characterization by a private

individual. Considering that such individuals may stand to gain privately from these public testing programs – either directly through the conduct of the tests, or indirectly as producers of competing products, for example – it would seem to be in the interest of fair and transparent government policy, as promoted specifically by our new administration, that the identity of nominators of chemicals to the testing program be made public.

**References**

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